ADMAIORA

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PU	Public	**X**
PP	Restricted to other programme participants (including the Commission Service)	
RE	Restricted to a group specified by the consortium (including the Commission Service)	
CO	Confidential, only for members of the consortium (including the Commission Service)	

Document History

Version	Date	Author	Summary of Main Changes
1	10/01/2023	Leonardo Ricotti, SSSA	First version of the Deliverable
2	16/01/2023	Leonardo Ricotti, SSSA	Request of contributions by the partners
3	30/01/2023	Paolo Cabras, IGT, Gina Lisignoli, IOR, Diego Trucco, Lorenzo Vannozzi, Alessia Bacci, SSSA	Filling requested contribution
4	31/01/2023	Leonardo Ricotti, SSSA	Integration, preparation and submission of the final Deliverable version

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1 Executive summary

ADMAIORA is a highly interdisciplinary, collaborative project. To achieve the final project objectives, intense efforts were invested in integrating the different technologies developed by the project partners, into viable and efficient solutions that could be tested in the final phase of the project. Integration is the focus of Task 5.4 and in this report the results obtained are described, in a qualitative way. Integration concerned three main domains of the project: (1) integration of cell-laden nanocomposite hydrogels and their ultrasound stimulation; (2) integration of stimulation brace, monitoring brace and IoT framework; (3) integration of handheld bioprinting tool. This Deliverable is publicly available; thus, only general indications and demonstration of the different integration procedures have been reported here, without entering technical details that should be considered confidential (due to possible future patenting, or publication in scientific papers).

2 Introduction

In the ADMAIORA project work plan, Task 5.4 is dedicated to technology integration. In this task, all partners collaborated in the final integration of the variegated technological components of the project, thus to guarantee the success of the final validation process.

The integration efforts concerned three domains:

- Integration of cell-laden nanocomposite hydrogels and their ultrasound stimulation (section 3)
- Integration of stimulation brace, monitoring brace and IoT framework (section 4)
- Integration of handheld bioprinting tool (section 5)

In this Deliverable, which will be publicly available, only general indications and demonstration of the different integration procedures will be shown, without entering technical details that should be considered confidential (due to possible future patenting, or publication in scientific papers).

3 Integration of cell-laden nanocomposite hydrogels and their ultrasound stimulation

The nanocomposite hydrogel hosting adipose tissue-derived stem cells (ASCs) is one of the key components of the ADMAIORA project. The Consortium focused its integration efforts on a hydrogel based on VitroGel® matrix, with 25 μg/mL of graphene oxide nanoflakes and 50 μg/mL of barium titanate nanoparticles, in which two million ASCs/mL were embedded (Figure 1).

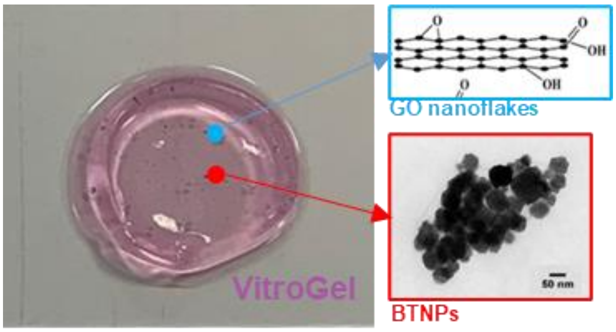

Figure 1: Photo of a nanocomposite hydrogel, with graphene oxide (GO) nanoflakes and barium titanate nanoparticles (BTNPs) embedded in a VitroGel® polymeric matrix.

The related integration efforts focused on optimizing the procedure for its preparation and sterilization, to make it repeatable, and testing the different properties that are crucial for its application in vivo (injectability, adhesion to the cartilage surface, etc.).

The nanocomposite hydrogel was stimulated with dose-controlled ultrasound waves, to identify the most efficient ultrasound parameters to boost the expression of cartilage-related markers and reduce inflammatory effects. The results obtained are part of a scientific paper, currently under revision. A preprint of this work, based on a close collaboration between SSSA, IOR, BIU, PLASMACHEM and IGT, is available on ResearchSquare:

https://assets.researchsquare.com/files/rs-2326785/v1/cde04f7d-2e3f-496b-8e56-0a723f291777.pdf?c=1670406690.

Some photos representing such activities are reported in Figure 2

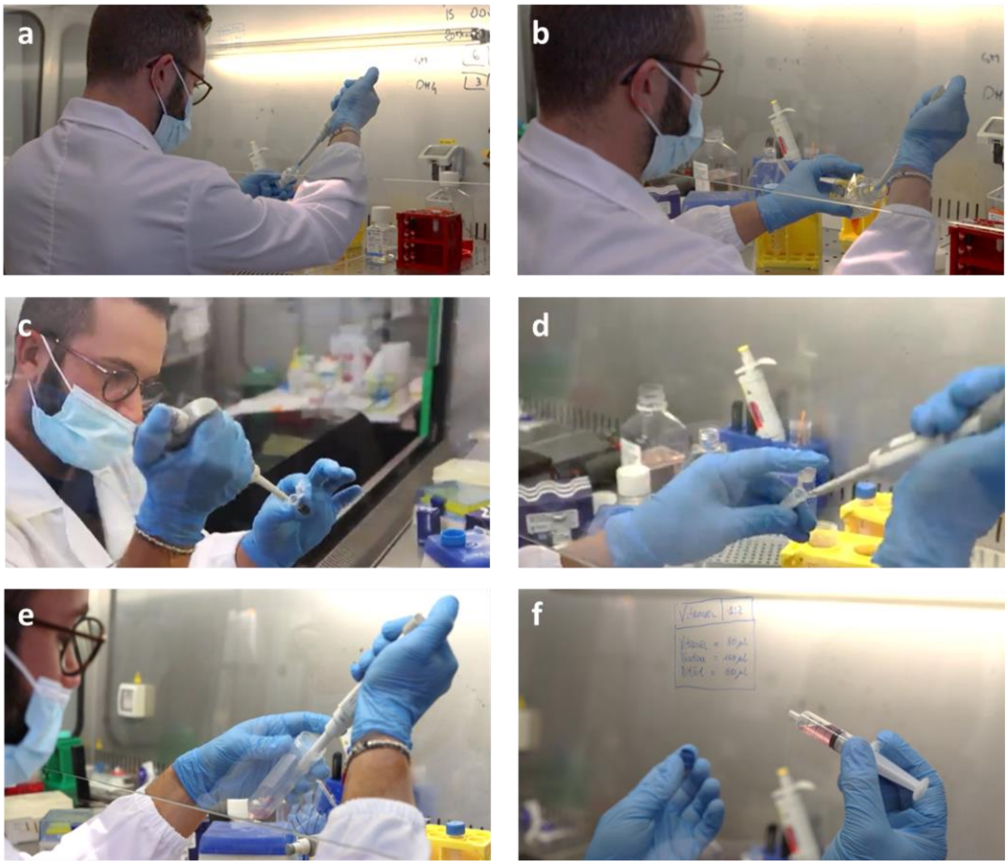

Figure 2. Depiction of the different steps of nanocomposite hydrogel preparation: a) Vitrogel-RGD® pouring into a conical tube, b) Dilution Solution addition, c) GO and d) BTNPs addition, e) mixing all components and f) cartridge loading for the next in vitro/in vivo treatments.

The ultrasound stimulation conditions that resulted the optimal ones to boost cartilage-relevant marker expression in the nanocomposite hydrogel, were also evaluated in terms of their translation in vivo. This was achieved through computer simulations, which predicted the correct exposure conditions, transducer placement, etc. to be used on rabbit models, on sheep models and on the human anatomy (Figure 4). Such integration effort required the close interaction between IGT, IOR and SSSA.

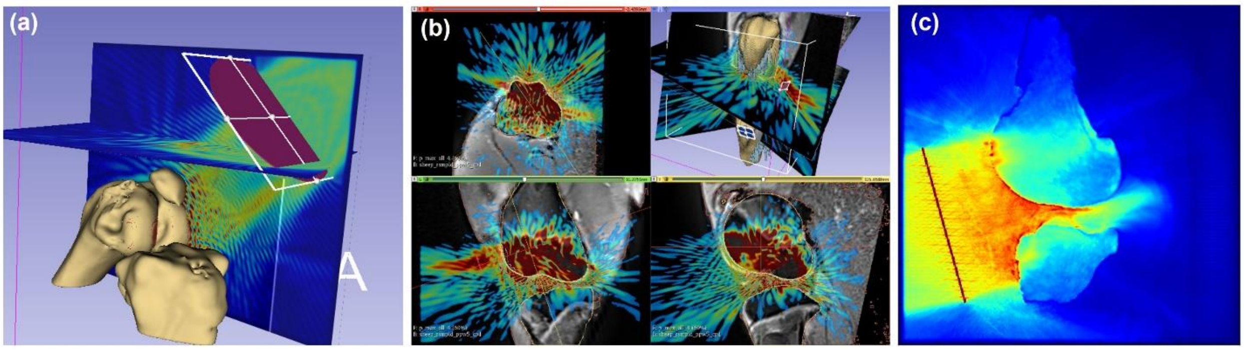

Figure 3. Example of pressure maps obtained from anatomy-specific 3D simulations: (a) rabbit knee, (b) sheep knee and (c) human knee. The disc represent the active surface of the transducer used for stimulation, the pressure map represent relative values and it is represented as a heat map (the hotter the colour the higher the pressure).

Further integration efforts on this topic concerned:

- the exploration of different polymeric matrices (e.g., methacrylated gellan gum, methacrylated gelatin, etc.), who may constitute an interesting alternative to VitroGel, in the future, and that generated results that will be published in future scientific papers;
- the exploration of the direct effects of ultrasound waves on macrophages, to minimize the production of inflammatory cytokines. The results obtained are part of a scientific paper, currently under revision;
- the exploration of the use of half concentration of nanomaterials in the hydrogel to verify LIPUS effect on chondrogenic differentiation that evidenced a lower cells differentiation.
- the exploration of the use of LIPUS for 28 days of chondrogenic differentiation instead of 10 days that evidenced worst results on chondrogenic differentiation.

4 Integration of stimulation brace, monitoring brace and IoT framework

In ADMAIORA, the ultrasound stimulation are thought to be provided to patients, in the future, through am ad hoc wearable brace that integrates ultrasound transducers. Another brace has been devised, with monitoring purposes (to check the advancement of the cartilage tissue healing, over time). Such a monitoring brace has been enriched, in the project course, with a series of other elements helping the overall monitoring procedure. A dedicated App running on a smartphone/tablet and an internet-of-things (IoT) framework complete this set of technologies (*Figure 4*).

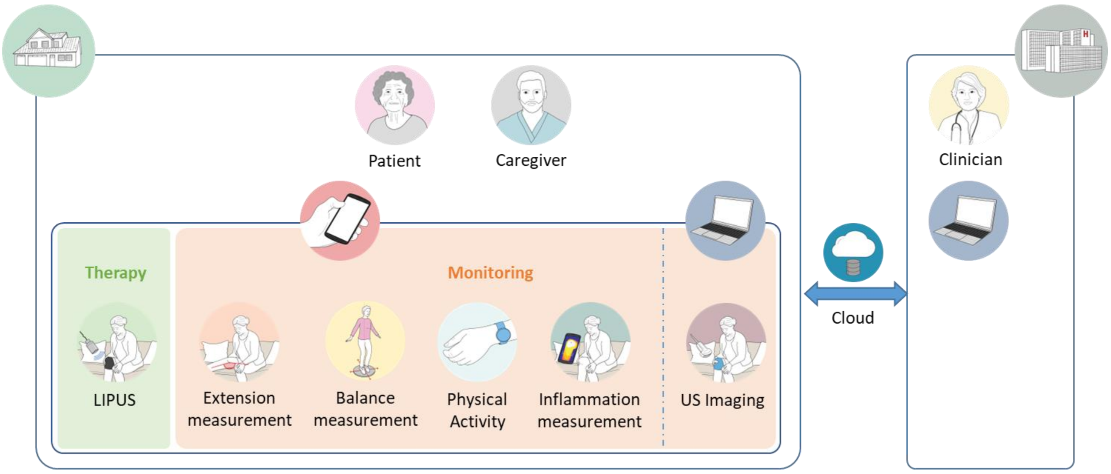

Figure 4: Technologies envisaged to monitor the cartilage healing process.

The integration of all these elements required an intense collaboration between IGT,HDW, SSSA and IOR, to guarantee the matching of the different components and to set the overall procedure to test them, in the usability tests by human volunteers (*Figure 5*, *Figure 6*, *Figure 7*).

Figure 5: Photo of a subject using the balance board, during an integration meeting.

Figure 6: Photo of a subject using the adjustable chair, during an integration meeting.

Figure 7: Photo of a subject wearing the stimulation brace, during an integration meeting.

Further integration efforts on this topic concerned:

- the exploration of raw radiofrequency (RF) data to extract information on the cartilage degradation process, using appropriate ex vivo cartilage tissues and correlating RF data with histological outcomes;

5 Integration of handheld bioprinting tool

A handheld bioprinting tool is an important component of the ADMAIORA paradigm, thought to deliver the nanocomposite hydrogel on the cartilage tissue in a non-invasive way, through an arthroscopic procedure (*Figure 8*).

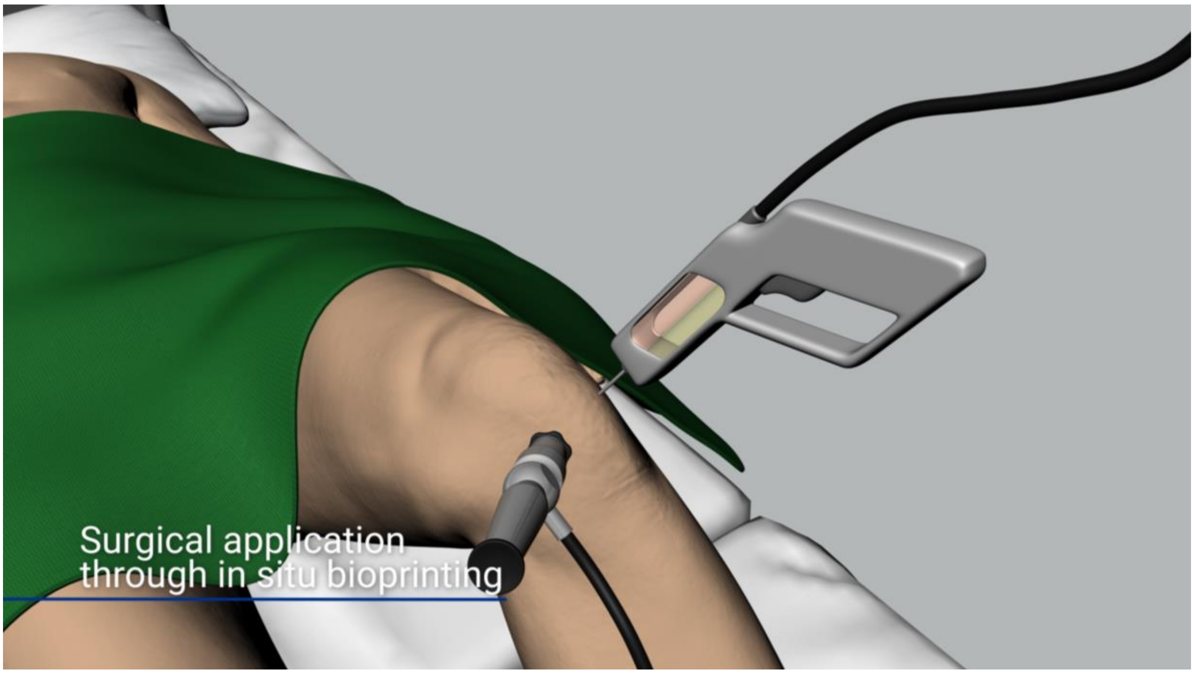

Figure 8: Depiction of the arthroscopic procedure for delivering the hydrogel on the cartilage site.

Different solutions were devised in the project course, grounded on different strategies. The integration of these solutions, to guarantee a platform that could be suitable for use on humans (and to be tested on human anatomy) required an intense collaboration between VIMEX, SSSA and IOR. Integration concerned both the integration of different platform components (e.g., a bendable tip developed by SSSA with a pneumatic extrusion system developed by VIMEX), but also the testing and fine parameter setting of such platforms with the cell-laden nanocomposite hydrogels developed in the project. Representative photos of these efforts are shown in Figure 9, Figure 10, Figure 11, Figure 12. The results obtained will be published in future scientific papers.

Figure 9: Pneumatic extrusion system testing during an integration meeting.

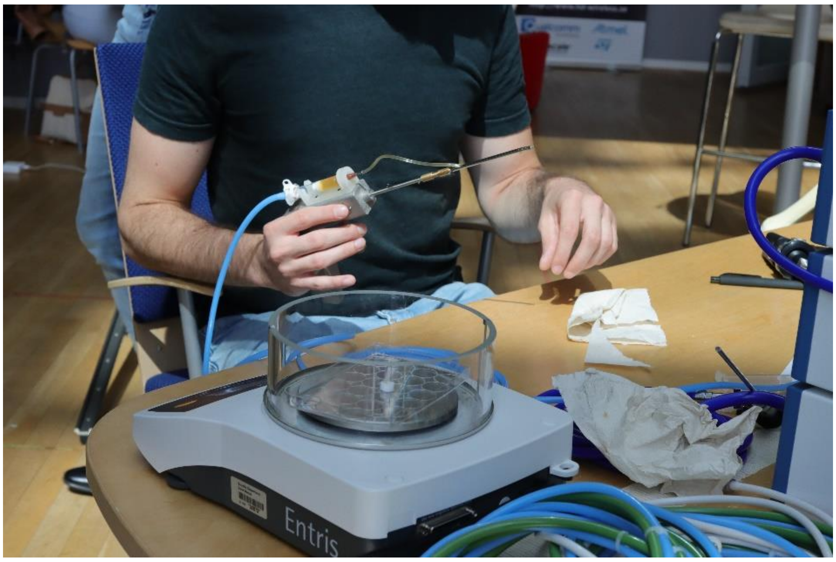

Figure 10: Test of the instrument delivery precision through a microbalance, during an integration meeting.

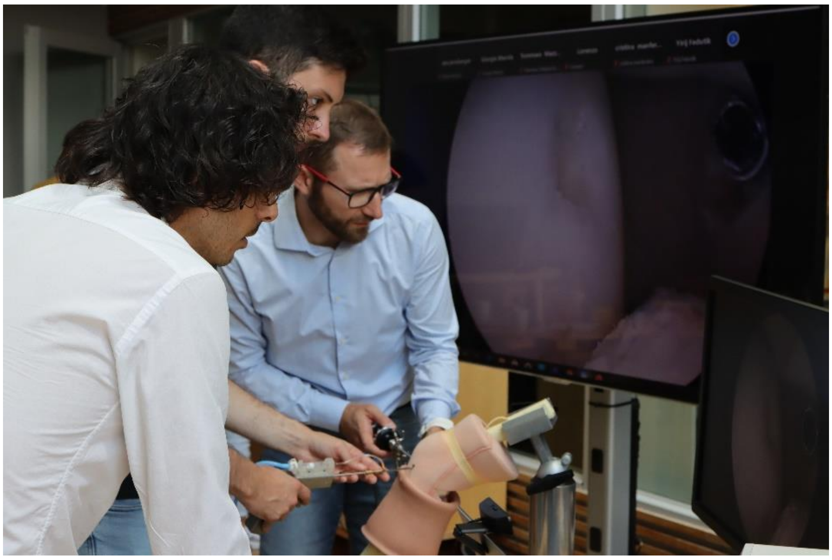

Figure 11: Handheld bioprinting tool testing during an integration meeting.

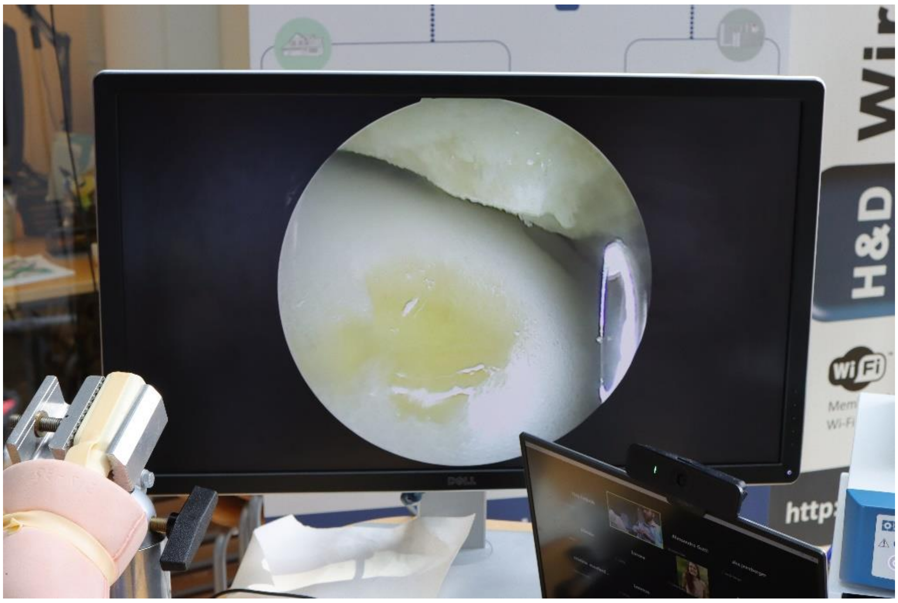

Figure 12: Delivery of the nanocomposite hydrogel in a knee phantom, during an integration meeting.

Further integration efforts on this topic concerned:

- the testing of cell viability after extrusion through the arthroscopic devices developed in the project, to verify their safety during the printing action;

6 Conclusions

Overall, the integration efforts carried out by the project Consortium were successful in the three domains of the project in which they were crucial, namely: (1) integration of cell-laden nanocomposite hydrogels and their ultrasound stimulation; (2) integration of stimulation brace, monitoring brace and IoT framework; (3) integration of handheld bioprinting tool. The ADMAIORA Consortium is now ready to enter the final validation phase for these technologies.